INTESTINAL ABSORPTION AND TRANSPORT IN *THYONE.*[1]
I. BIOLOGICAL ASPECTS

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During the first three decades of this century several investigators reported controversial observations about the digestion of food and the intestinal transport of nutrients in holothurians. These reports have been reviewed by Anderson (1966). Briefly, Enriques (1902), Oomen (1926), and Schreiber (1931) reported that the intestine of *Holothuria* is impermeable to a variety of sugars, salts, and dyes. These authors proposed that the distribution of nutrients from the intestinal lumen to the various tissues of the body is mediated by wandering coelomocytes which shuttle between the intestinal lumen and the different tissues and body fluids of holothurians. This view was based only upon the observation that coelomocytes are found in histological preparations of all holothurian tissues. While amebocytic transport of this type probably plays a substantial role in nutrient distribution among animals which do not possess well differentiated digestive tracts, it is difficult to accept the contention that such a mode of nutrient transport is effectively operative in echinoderms with well developed digestive systems. Some support for the coelomocyte theory might be adduced from the observation of direct uptake of dissolved sugars and amino acids from surrounding sea water. This has been demonstrated for various echinoderms by Koller (1930), Stephens and Schinske (1961), and Ferguson (1967). However, this mode of nutrition does not appear to supply these animals with a significant number of calories. Echinoderms are known to feed on a variety of materials in nature ; their digestive tracts are usually found to be packed with specific materials. When deprived of solid or particulate food, they starve ; there is a rapid drop in metabolic rate and body reserves. For the pertinent data see Farmanfarmaian (1966), Giese (1966a), Holland, Giese and Phillips (1967) and Lawrence, Lawrence and Holland (1965).

In studies on the transport of nutrients in echinoids, Farmanfarmaian and Phillips (1962) demonstrated that galactose is directly transported across the intestinal wall without appreciable involvement of coelomocytes. Ferguson (1964) used C^{14} labeled glucose and amino acids and reached the same conclusion for asteroids. The holothurian digestive tract is well developed and usually longer than those of echinoids and asteroids (Choe, 1962). It seemed unlikely that this extensive intestine should be impermeable to digested nutrients as was claimed by the early authors. These considerations led to preliminary investigations of the transport of glucose across the intestine of *Leptosynapta* by D'Agostino and

Farmanfarmaian (1960); of *Holothuria* by Farmanfarmaian (1963); and of *Thyone briareus* by Rundles and Farmanfarmaian (1964). The intestinal wall of all three species were shown to be permeable to glucose but these exploratory studies did not reveal any details of the mechanisms of nutrient absorption and transport in holothurians. Furthermore, the cellular mechanisms involved in the intestinal transport of most invertebrates have not been adequately investigated. By contrast, the mammals have been extensively studied in this respect. For recent reviews of the mammalian literature see Wilson (1962), Wiseman (1964), and Crane (1968).

The above considerations prompted a detailed *in vivo* and *in vitro* investigation of the intestinal absorption and transport of nutrients in *Thyone briareus*. Since this organism is not a standard laboratory animal, much of the biological information relevant to the mode of nutrition and the mechanisms of absorption and transport had to be determined as part of the present project. This paper is devoted to the presentation of this biological background.

MATERIALS AND METHODS

Animals

Specimens of *Thyone briareus*, weighing 25–50 g, were obtained from the Supply Department of the Marine Biological Laboratory on the day of collection. All the animals used in these investigations were collected between June 10 and September 10. The animals in each collection were kept at about 20° C in separate shallow tanks of running sea water without special feeding. To circumvent the effects of the duration of captivity on various experiments, only animals from the same collection, and with similar background, were used for comparable experiments.

The dissection procedure was as follows: The animal was lightly anesthetized in 7% $MgCl_2$ tap water solution for 40 minutes and thoroughly rinsed in sea water. The relaxed animal was placed on a shallow dissecting pan and a postero-anterior incision made along the convex curvature of the body wall. The incision was extended laterally near the anterior and posterior ends and the body wall was pinned out under 1.5 cm of sea water. Under these conditions the animal was completely immobilized although the effect of anesthesia had worn off. In these preparations vital functions such as the movements of the gut and the branches of the water lungs could be observed for several hours at 20° C.

Chemical analyses

Glucose was measured colorimetrically by the glucose oxidase method (Glucostat and Glucostat Special from the Worthington Biochemical Corporation, Freehold, New Jersey). A Bausch and Lomb Spectronic 20 was used for this purpose. The precision of the method was ±2% in the range of 5–30 µg per sample. Total carbohydrates were measured according to the anthrone method of Koehler (1952) and the values are expressed in terms of glucose equivalents. Glycogen was purified according to Be Miller (1965) and analyzed by the anthrone method.

For thin layer chromatography (TLC) of sugars, Eastman cellulose sheet number 6064 and developing apparatus number 6071 were used. Solutions containing 1–2 µg of sugar were spotted on the sheet about 3 cm apart and the

chromatogram was developed in ethyl acetate:pyridine:water (120:50:40 vol.) at 23° C. The TLC sheets were visualized by the AgNO₃ and NaOH dip method of Smith (1960). For desalting prior to chromatography, 1–2 volumes of wet ion exchange resin (Rexyn 300-H-OH Research Grade, Fisher Scientific Company) was mixed with the sample in a centrifuge tube for 2–3 minutes. The fluid was aspirated and the resin washed twice with distilled water and the washes added to the aspirated fluid. This procedure gave 70–75% recovery of sugars without detectable salts. Further washes increased the recovery to better than 90%. When desired the desalted fluid was concentrated under vacuum at 40° C.

Total lipid was determined by a modification of the method of Freeman, Lindgren, Ng and Nichola (1967). Tissue samples of 4–5 g fresh weight were extracted in 50 ml methanol at 65° C for 10 minutes. At room temperature, 100 ml of chloroform was added to the mixture and the extraction was continued under constant stirring for 4 hours. The extract was filtered and passed through a 10 cm column of Unisil (silicic acid). This was followed by elution with 100 ml of 95% methanol and 5% water to obtain all the phospholipids. The eluates were combined and concentrated. Aliquot portions were dried in tared glass tubes under nitrogen and weighed.

Water content of intestinal tissues were determined as the difference between the wet weight and the constant dry weight measured by a Mettler analytical balance. The wet weight was measured after the tissue was allowed to drain on a piece of Whatman No. 1 filter paper for 30 seconds.

For pH measurements a Beckman Zeromatic pH meter was employed.

Histological preparations

Tissues were fixed in Bouin sea water fixative. Sections of 10 μ thickness were stained with iron hematoxylin or Mallory's triple stain. For electron microscopy tissues were fixed in 6% glutaraldehyde, postfixed in osmium tetroxide, and embedded in maraglass or Epon 812. These sections were cut at 0.5 μ and a few were stained with methylene blue Azure B for light microscopy.

RESULTS

Ecological observations

In the Woods Hole region *Thyone briareus* is found partially buried among the eel grass in sallow bays. In the contracted state the size of the mature specimens may vary from 4 to 5 cm in length. The ambient temperature of its environment, as reported by Mr. John Valois, Chief Collector of the Supply Department, varies from 1° C in winter to 22° C or more in the summer. The sexes are separate and the gametes are usually spawned out by the end of June. These animals are particle feeders, using their tentacles as a filtering mechanism. Feeding activity has not been observed during winter when *Thyone* appears to be in a state of hibernation.

In the laboratory the animals survive well for more than three months at about 20° C in shallow tanks of running sea water without special feeding. Attempts at controlled feeding with sea water species of *Chlorella* have failed. *Thyone* can

tolerate salinities as low as 50% sea water and as high as 120% sea water for 24 hours or more.

During the summers, when the investigations to be reported here were carried out, the contents of the digestive tract largely consisted of particles of the eel grass *Zostera*. When animals are brought into the laboratory, the gut is emptied within a week and the feces consist of greyish masses of unrecognizable amorphous materials which disintegrate at the bottom of the tank.

The carbohydrate composition of various species of *Zostera* has been determined by Dudkin, Shkantova, Skornyakova and Lemle (1964) and Williams and Jones (1964). This sea grass is known to contain glucose, fructose, galactose, xylose, arabinose, rhamnose, and apiose. The natural gut fluid of *Thyone* was analyzed for glucose in the following manner. The intestinal contents taken from 15 animals (freshly collected during July) were pooled and centrifuged at 5° C. The iced supernatant was deproteinized with $Ba(OH)_2$ and $ZnSO_4$, decolorized with norite, and the pH adjusted to 7.0. Samples were analyzed by the highly purified glucose oxidase (Glucostat Special). The glucose content was 33.2 $\mu g/ml$ of the original intestinal fluid. Samples analyzed for total carbohydrates by the anthrone method gave a glucose equivalent value of 66.7 $\mu g/ml$ for freshly collected animals and 31.2 $\mu g/ml$ for animals starved for 24 hours in the laboratory. These results serve to indicate the general level of dissolved sugars in the natural intestinal fluid of *Thyone*.

Anatomical and histological observations

Figure 1 illustrates a freshly dissected digestive tract laid out in an approximately natural disposition. Various nomenclatures have been applied to the different regions of the digestive tract of holothurians (Fish 1967a). In the case of *Thyone*, the digestive tract consists of an oropharynx, a short esophagus which goes through the calcareous aquapharyngeal bulb, a yellowish bulbous stomach, and a long brownish red intestine which terminates in the cloaca. The intestine constitutes 3–4% of the animal fresh weight. For the purpose of the physiological studies, the intestine has been divided into zones which are anatomically recognizable (Fig. 1). These zones are:

First loop—from the posterior end of the stomach to the last posterior cross connecting hemal sinus of the hemal network.

Clear zone—the segment between the first and second loop which has no interconnecting hemal sinuses to the hemal network.

Second loop—the segment between the first (anterior) and the last (posterior) cross connecting hemal sinuses of the network on the ascending part of the intestine.

Cloacal segment—the remaining part of the intestine which terminates in the cloaca and has been referred to as the large intestine in the literature. The intestine of animals which have eviscerated and are in the process of regenerating the intestine does not precisely conform to the above anatomical description.

Histological sections of the intestine were examined by light microscopy and low magnification electron microscopy.

From the lumen outward, the intestinal wall typically consists of tall (100–180 μ long) mucosal epithelial cells with a brush border (Figs. 2 and 3). These cells
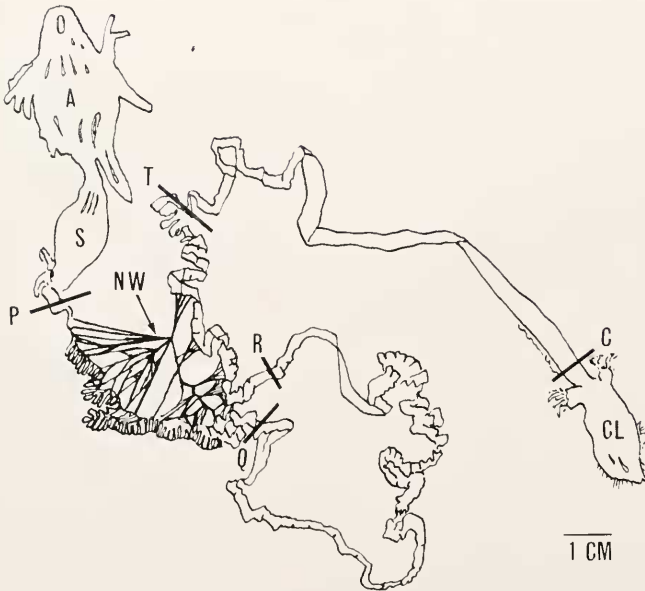
FIGURE 1. Freshly dissected digestive tract of *Thyone* laid out in approximately natural size and disposition. O-oropharynx; A-aquapharyngeal bulb containing the esophagus; S-stomach; P to Q-first loop; Q to R-clear zone; R to T-second loop; T to C-cloacal segment; CL-cloaca; NW-net work of hemal sinuses interconnecting the first and the second loop.
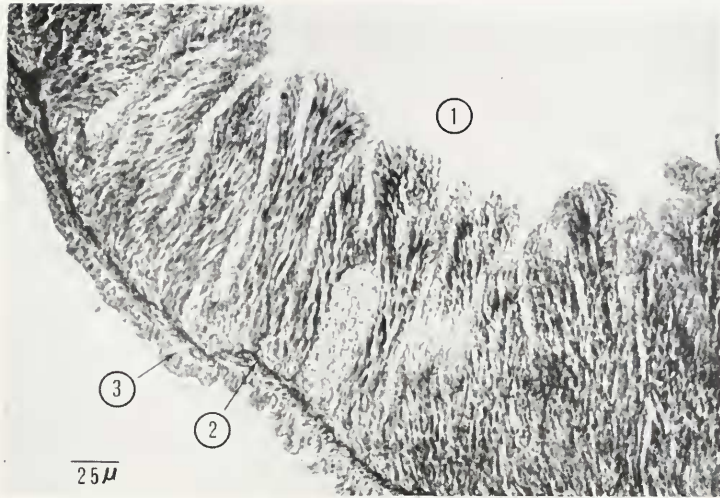
FIGURE 2. Cross section near the anterior end of the first loop showing the brush border of the tall mucosal epithelium extended into the lumen (1); circular muscle bands (2); and the flat serosal epithelial cells (3). Fixed in Bouin and stained with iron hematoxylin.

are loosely organized into villus like structures which make up the bulk of the intestinal tissue (Fig. 2). At the base of the epithelial cells is the connective tissue-lacunar area (10–30 μ wide) with mixed connective tissue fibers and a fluid which has the same staining properties as the clear material in the mesenterial sinus (Fig. 4). This fluid is in communication with the hemal sinuses (Figs. 4 and 5). The connective tissue-lacunar areas invade the base of the "villi" and break up into fine channels (Fig. 4), somewhat akin to the lymphatic channels of the vertebrate villi (Bell and Farmanfarmaian, 1967). Toward the outside there is a longitudinal muscle layer followed by a circular muscle layer (Fig. 5). These muscles are particularly prominent in the cloacal segment of the intestine (10–15 μ). Peripheral to the muscles is a thin connective tissue layer and finally the flat serosal epithelium (10–30 μ) bearing a few observable cilia (Fig. 5).

The intestine is attended by a mesenterial and an antimesenterial hemal sinus along its length (Figs. 4 and 5). The side channels of these sinuses invade the serosal epithelium and open into the connective tissue-lacunar area below the mucosal epithelium. There are numerous interconnecting hemal sinuses forming a network between the antimesenterial sinus of the first loop and the second loop (Fig. 1). The anatomical relation of the hemal sinuses to the intestine would seem to indicate that the hemal sinuses provide for a functional circulation of the absorbed nutrients. In surviving preparations, which were dissected as described in a previous section, there was no observable unidirectional Harveyan flow in these sinuses. Weak local contractions provided for gentle ebb and flow movements of the viscous contents of the channels. By contrast, vital functions such as the movement of sea water in and out of the branches of the water lungs, the circulation of the hemoglobin-containing hemocytes in the ampullae of the podia, and the peristalsis of the gut were all clearly observable for several hours in such a
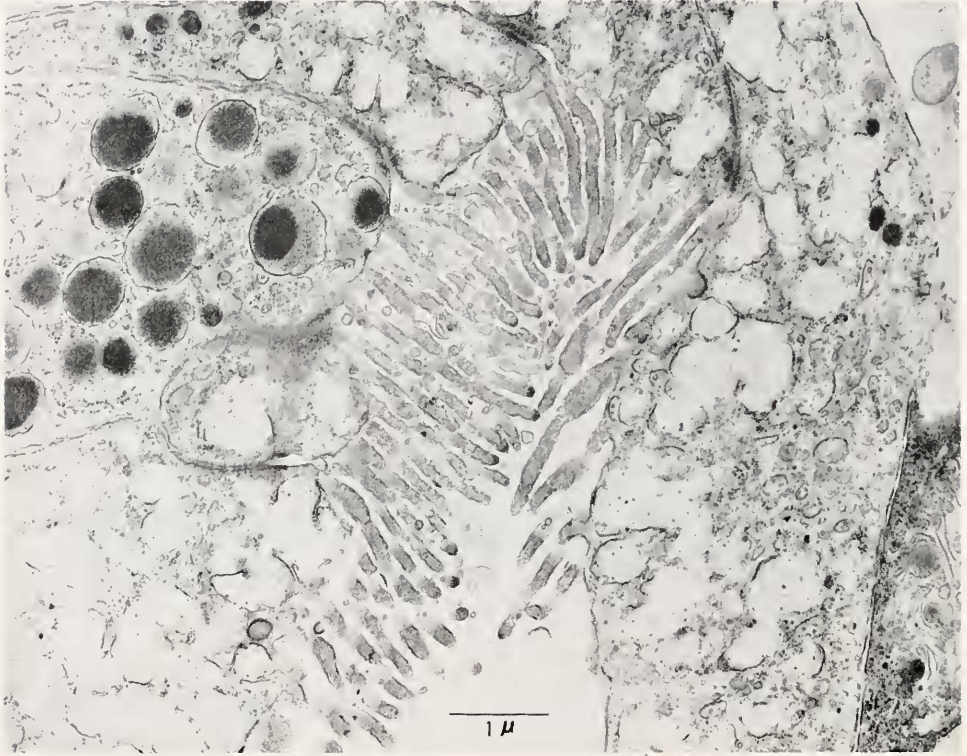
FIGURE 3.   Electron micrograph showing the brush border "microvilli" of the mucosal epithelial cells.   Photograph kindly provided by Dr. Allen Bell.   See text for methods of preparation.

preparation.   There are no functional hearts of any nature in holothurians.   In some species, however, more prominent local contractions can be observed in the mesenterial sinuses.   For example, in preparations of *Leptosynapta* a vigorous churning motion was observed in the mesenterial sinus which is tightly applied to the wall of the gut.   This motion, however, was caused by the peristaltic waves of the intestine and had the same rhythmicity.   The viscous yellowish fluid in the mesenterial sinus moved back and forth and there was no evidence for its circulation. When the hemal sinuses of *Thyone* and *Leptosynapta* were cut, the hemal fluid did not spurt.   Either there was no flow from the cut end or only a small amount of the viscous yellowish fluid slowly leaked out.   The author has also examined many other American and European echinoderms in this connection.   In no case has he observed a unidirectional Harveyan circulation in the hemal system of echinoderms.

The total weight of the full (undrained) network sinuses in a 50 g *Thyone* is about 2 mg.   This represents roughly 25% of the entire hemal system of the animal and indicates the low capacity of this system, 0.02% of the body weight.

FIGURE 4. Cross section of the first loop cut at 0.5 μ. The side channel of the hemal sinus (1) is shown entering the serosal epithelium. Small channels of the connective tissue-lacunar area (2) penetrate between the bases of the mucosal epithelial cells. Lumen (3). Photograph kindly provided by Dr. Allen Bell. See text for methods of preparation.

Hemal and perivisceral fluids

It has not been possible to obtain samples of the content of hemal sinuses without contamination and in adequate amounts for analysis. When a portion of fresh hemal sinus is smeared on a microscope slide, examination shows that it contains yellow, brown, and black refractile spherules. These bodies show autofluorescence under ultraviolet light and in this respect are similar to the lipofuscin granules described by Goldfischer, Villaverde and Forschirm (1966). Histochemical studies of the hemal sinuses of *Thyone* (Hugh Y. Elder, personal communication) showed that the clear fluid is PAS positive but Alcian blue and hemalum negative. These reactions indicate a large amount of dissolved neutral polysaccharides or possibly non-sulphated mucopolysaccharides or glycoproteins. The lining of the sinuses, however, contains acid mucopolysaccharides. In addition to the clear fluid and lipofuscin spherules, coelomocytes are always observed in various parts of the hemal sinuses.

The perivisceral fluid occupies the large body cavity of holothurians and bathes all the internal organs. A large specimen of *Thyone* (40–50 g fresh) contains about 10 ml of this fluid. The perivisceral fluid contains a variety of coelomocytes (Hetzel, 1965; Endean, 1966). The ionic composition and the osmotic pressure of this fluid are essentially the same as those of the surrounding sea water (Binyon, 1966).

For chemical analysis, perivisceral fluid was obtained by a direct incision of the body wall of *Thyone*. The fluid was pooled from 5 or more freshly collected animals in July. The pooled sample was deproteinized by 10% TCA, its pH adjusted to 7.0 and centrifuged at 5° C. The supernatant was concentrated in a
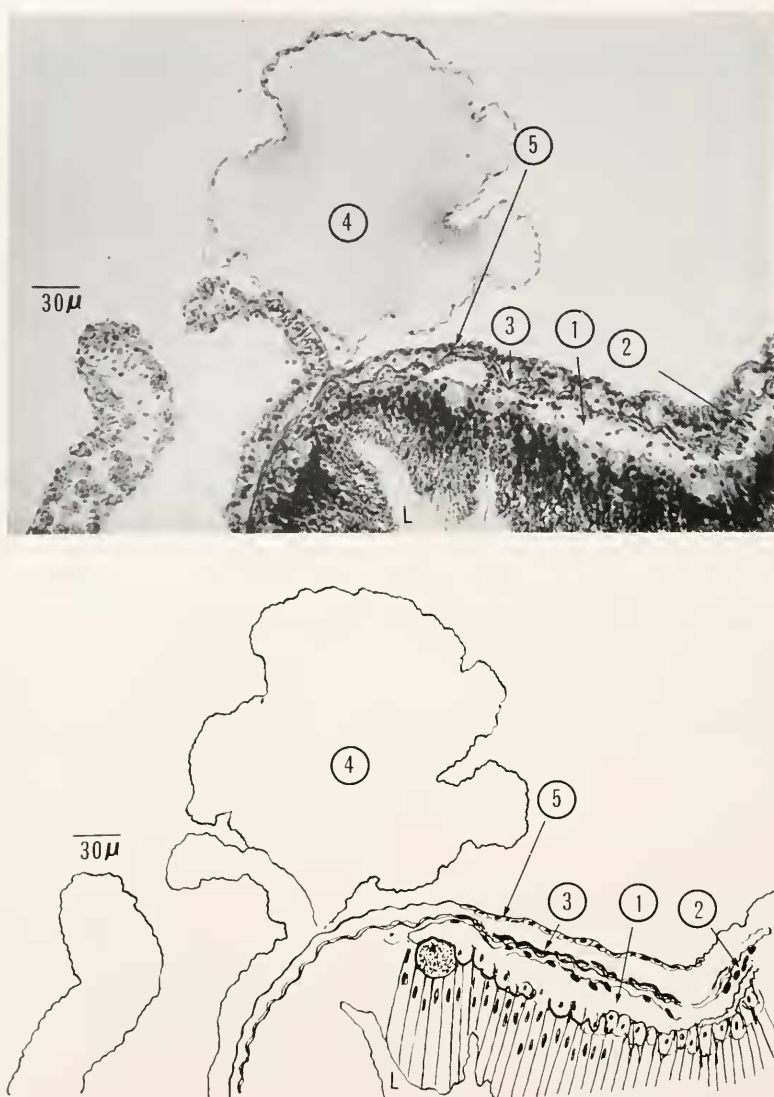
FIGURE 5. Cross section of the clear zone showing: connective tissue-lacunar area (1), longitudinal muscle bands (2), circular muscle bands (3), clear viscous material in the mesenterial sinuses (4), serosal epithelial cells (5), and lumen (L). Fixed in Bouin and stained with Mallory's triple stain.

Buchler evaporator at 40° C. Samples of the concentrates were analyzed by the anthrone method for total carbohydrates and by Glucostat Special for glucose. The values obtained for total carbohydrates were 6.3 and 7.4 μg/ml and those for glucose were 1.2 and 1.5 μg/ml of original fluid. Several sugars were identified by thin layer chromatography of the concentrates. These were glucose, galactose,

xylose, and trehalose. Thus, the perivisceral fluid of *Thyone* contains an appreciable amount of dissolved carbohydrates in nature and these include several of the sugars found in the natural diet of the animal.

The pH of the perivisceral fluid measured outside of the body of a number of freshly collected animals varied between 7.3–7.8.

Chemical composition of the intestinal tissue

Intestinal segments from the first loop and the clear zone were removed from freshly collected *Thyone* and both the mucosal and serosal surfaces were washed with cold filtered sea water. These tissues were homogenized in iced 10% TCA. Aliquot portions of the whole homogenate were used to determine total carbohydrate. Other aliquots were neutralized, decolorized by norite, and centrifuged. The supernatant was analyzed for glucose by Glucostat Special. For animals collected in July mean value of total carbohydrate for the first loop was 10.3 mg/g wet (SEM ± 0.5; N = 9) and for clear zone was 13.9 mg/g wet (SEM ± 0.6; N = 9). In the August collection corresponding values were 12.0 and 16.6 mg/g wet. In all of these segments, the free glucose content was below the sensitivity (20 μg/g wet) of the method used. However, by thin layer chromatography it was possible to identify glucose, galactose, and trehalose qualitatively. It appears, therefore, that free sugars occur in small amounts in the intestinal tissue of animals a few hours after collection.

The glycogen content of the first loop and clear zone segments (pooled from 4 animals freshly collected in August) was determined by the anthrone method after two purifications according to Be Miller (1965). The values obtained were 2.7 and 6.2 mg/g wet (or 12.3 and 28.2 mg/g dry) for the first loop and the clear zone, respectively. Glycogen constitutes 22.8% and 37.2% of the total carbohydrate of the respective intestinal segment. The occurence of glycogen, acid mucopolysaccharide, neutral mucoproteins and glycoproteins, and possibly glycolipids has been confirmed histochemically (Hugh Y. Elder, personal communication).

For total lipid determination segments of the first loop and clear zone were separately pooled from 10 animals freshly collected in July. The values obtained were 45.4 and 48.8 mg/g wet (or 207 and 222 mg/g dry) for the first loop and clear zone, respectively.

The mean tissue water for the combined first loop and clear zone segments was 78% (SEM ± 0.48; N = 34) of the wet weight.

DISCUSSION

The digestive tract of *Thyone* is anatomically and histologically well adapted to the digestion and absorption of carbohydrates found in the particles of the eel grass *Zostera* which constitute a large portion of the normal diet of the animal during the summer. The length of the gut (about 50 cm in a 15 cm animal) and the peristaltic and tonic movements of the intestine are appropriately suited to continuous filter feeding. Such an arrangement provides for a large reserve of absorptive surface so that absorption can continue as the intestinal contents are moved distally. The relative weight of the alimentary tract (3–4% of body

weight) is appreciably higher than mammals, about 2.3% (Long, 1961). This is probably due to the absence of liver, hepatopancreas, or hepatic caeca in *Thyone*. Most of the hepatic storage functions appear to be retained in the intestinal wall. The tall mucosal epithelial cells constitute the bulk of the intestinal tissue. These cells are 5–10 times taller than the columnar epithelial cells of the mammalian intestine (Bloom and Fawcet, 1962). The luminal borders of the mucosal epithelial cells are invested with an extensive brush border whose "microvilli" appear similar to those of the mammals both in structure and size; approximately 1 μ in length and 0.1 μ in width (Trier, 1968). By analogy to the mammals, the structural evidence indicates that the "microvilli" constitute the normal site for the absorption of nutrients. There is no basis for rejecting this extensive brush border as the absorptive site in favor of a relatively few coelomocytes which are observed in the intestinal tissue. In three different species of holothurians it was experimentally demonstrated that the transfer of glucose from the mucosal to the serosal side of *in vitro* intestinal segments proceeds in the absence of coelomocytes (D'Agostino and Farmanfarmaian, 1960; Farmanfarmaian, 1963; Rundles and Farmanfarmaian, 1964). Comparable data were provided for the intestinal transfer of nutrients in echinoids and asteroids (Farmanfarmaian and Phillips, 1962; Ferguson, 1964). Thus, the coelomocyte theory proposed by the early investigators (Enriques, 1902; Oomen, 1926; Schreiber, 1931) is rejected.

In the mammal, absorbed nutrients are rapidly transferred from the intestine to the lymph and the blood, and from there to the liver and other organs. In *Thyone*, the first site of storage and chemical conversion is the mucosal epithelium. This view is supported by the following lines of evidence: (a) There is no liver-like organ in this animal. (b) The carbohydrate and lipid content of the intestinal tissue indicates that this organ is an important site of reserve material. The total carbohydrate of the intestinal tissue of *Thyone* is about 1/3 and the glycogen about 1/10 of the glycogen values reported by Stimpson (1965) for the liver of rat and goldfish. Nevertheless, this is an appreciable amount of carbohydrate reserve for *Thyone* which has a continuously dependable external source of carbohydrates during the feeding season. The lipid content of the intestine is about 22% of the dry weight while the lipid content of the whole animal is only 1% of the total dry weight (Giese, 1966b). In another holothurian, *Parastichopus*, the lipid content of the intestine was appreciably higher than all other organs (Giese, 1966a). Therefore, the intestinal tissue appears to be the most important site of lipid storage and possibly lipid synthesis. (c) The sea urchin, *Strongylocentrotus purpuratus* is similar to *Thyone* in that it also lacks a liver-like organ and the mucosal epithelial cells constitute the main part of the intestine. When C^{14}-labeled discs of the alga, *Iridaea* (which is equally labeled in its reserve galactose and glycerol) were fed to this sea urchin, the specific activity of the proximal intestine was consistently higher than all other tissues for 7 days after feeding (Farmanfarmaian and Phillips, 1962). The level of activity gradually reduced as the animal was starved and the stored material was mobilized from the intestine for utilization by the other tissues. Lawrence, Lawrence, and Giese (1966) confirmed the above observations in the same sea urchin by reporting that the relative gut size decreases with starvation, primarily as a result of reduction in lipid content. The carbohydrates also declined. Realimentation arrested the decrease and replenished these

reserves in the intestine. (d) Finally, *in vivo* experiments on the intestinal absorption and transport of glucose presented in the following paper further support the view that the intestinal tissue is the first site for the storage of absorbed sugar.

The transport of nutrients from the alimentary canal to the other tissues of echinoderms has been a subject of much controversy. Most members of this phylum have three body fluid systems, namely, the water vascular system, the hemal system, and the perivisceral fluid. The last two have been implicated in the transport of nutrients. The evidence favoring the hemal system is based upon anatomy and the observation of local contractions of the sinuses of this system. By contrast nearly all the experimental evidence from studies of respiratory gas transport and nutrient transport indicates that the perivisceral fluid is the functionally effective circulatory fluid of adult echinoderms (Farmanfarmaian, 1968). Among the members of this phylum, the holothurians have the most extensive and the largest hemal systems. Nevertheless, the capacity of the hemal system is negligible when it is compared to the perivisceral fluid or the circulatory fluids of comparable animals. In *Thyone*, the weight of the system with its fluid contents is only 0.02% of the total body weight. By contrast, the perivisceral fluid is approximately 20% of the body weight. The blood volume of poikilotherm vertebrates and invertebrates, whose metabolism is of the same order of magnitude as *Thyone*, is 2–80% of body weight (Prosser and Brown, 1961; Farmanfarmaian, 1966). Therefore, there is good agreement between the capacity of the perivisceral fluid of *Thyone* and the well known circulatory fluids of other metabolically comparable animals. If the circulation in the hemal system were efficient, it might still be assumed that the rapid turn over of a few milligrams of hemal fluid may effectively contribute to the distribution of nutrients since the hemal system is so intimately related to the intestine. In the absence of any kind of a heart or unidirectional valves, there is no Harveyan circulation in this system. Only weak local pulsations of 4–5 beats per minute have been observed in these sinuses (Kawamoto, 1927; Wyman and Lutz, 1930; Prosser and Judson, 1952). On the basis of the available evidence, it is not possible to assign a definite function to the hemal system; it may be vestigial, have an embryonic function, or be involved in the elaboration and slow distribution of some special compounds. The *in vivo* experiments reported in the following paper provide direct evidence for consideration of the perivisceral fluid as the functional circulatory fluid of *Thyone*.

The digestive enzymes of *Thyone* have not been investigated. The simultaneous presence of several sugars including glucose and galactose in the diet, intestinal fluid, intestinal tissue, and the perivisceral fluid indicates that the digestive tract contains the necessary carbohydrases for the digestion of plant particles in the diet. Related species have been investigated in this respect and contain various carbohydrases, proteases and lipases (Fish, 1967b; Choe, 1962). Glucose appears to be the most important sugar in the economy of *Thyone* since it occurs in appreciable quantities in the diet and constitutes half of the total dissolved carbohydrate in the natural intestinal fluid. The concentration of glucose in the intestinal fluid is 20 times higher than the perivisceral fluid. This implies that in nature the transport of glucose across the intestinal wall does not proceed against a chemical gradient. Further studies on the absorption and transport of glucose by the intestine of *Thyone* are reported in the following paper.

I am indebted to Drs. J. M. Anderson and N. Holland for reading and commenting upon this paper.

Summary

Several investigators have proposed that the transport of nutrients from the intestine to the tissues of holothurians is mediated by wandering coelomocytes. These and other authors have also implied that hemal system is a functional circulatory system in adult holothurians. On the basis of the present studies, these views are rejected. Evidence for the direct absorption of glucose by the intestinal epithelium and its transmural transport into the perivisceral fluid is presented. The perivisceral fluid is the main circulatory medium for gaseous exchange and nutrient transport to the internal tissues. Ecological, anatomical, and histological information pertinent to the study of intestinal transport mechanisms is presented. In addition to digestive and absorptive functions, the intestinal tissue serves as the storage site for relatively large quantities of carbohydrate and lipid reserves.

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